

# Direct observation of a hydrophobic bond in loop-closure of a capped $(-\text{OCH}_2\text{CH}_2-)_n$ oligomer in water

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The small  $r$  variation of the probability density  $P(r)$  for end-to-end separations of a  $-\text{CH}_2\text{CH}_3$  capped  $(-\text{OCH}_2\text{CH}_2-)_n$  oligomer in water is computed to be closely similar to the  $\text{CH}_4 \cdots \text{CH}_4$  potential of mean force under the same circumstances. Since the aqueous solution  $\text{CH}_4 \cdots \text{CH}_4$  potential of mean force is the natural physical definition of a primitive hydrophobic bond, the present result identifies an experimentally accessible circumstance for direct observation of a hydrophobic bond which has not been observed previously because of the low solubility of  $\text{CH}_4$  in water. The physical picture is that the soluble chain molecule carries the capping groups into aqueous solution, and permits them to find one another with reasonable frequency. Comparison with the corresponding results without the solvent shows that hydration of the solute oxygen atoms swells the chain molecule globule. This supports the view that the chain molecule globule might have a secondary effect on the hydrophobic interaction which is of first interest here. The volume of the chain molecule globule is important for comparing the probabilities with and without solvent because it characterizes the local concentration of capping groups. Study of other capping groups to enable X-ray and neutron diffraction measurements of  $P(r)$  is discussed.

Hydrophobic interactions are central to supermolecular self-assembly in aqueous solutions<sup>38–43</sup>, including the folding of soluble globular proteins.<sup>44</sup> A surprising development of recent years is that for entropy-dominated hydrophobic solubilities we now have statistical mechanical theories that are fully defensible on a molecular scale, exploiting all available molecular-scale data.<sup>45–47</sup> In contrast, our understanding of the statistical solvent-induced forces between neighboring small hydrocarbon molecules in water — hydrophobic interactions — has *not* experienced the correspondingly conclusive progress<sup>48</sup> despite intense<sup>49,50</sup> computational effort.

The principal impediment to progress in understanding hydrophobic interactions is the lack of an accessible direct observation of a primitive hydrophobic bond, such as the  $\text{CH}_4 \cdots \text{CH}_4$  potential of mean force. Because simple hydrophobic species, such as  $\text{CH}_4$ , are only sparingly soluble in water, direct observations of primitive hydrophobic interactions are difficult to achieve; instead the influence of hydrophobic interactions is inferred in more complex systems — protein folding being the foremost example.<sup>44</sup> Where limited thermodynamic evaluation of hydrophobic interactions has been experimentally achieved for soluble, but more complex hydrophobic species with higher aqueous solubilities,<sup>51,52</sup> those experiments set-off a substantial modeling effort that has not untangled this complicated issue.<sup>48</sup>

Here we show (FIG. 1) that the probability density  $P(r)$  of end-to-end separations of a  $-\text{CH}_2\text{CH}_3$  capped  $(-$

$\text{OCH}_2\text{CH}_2-)_n$  oligomer in water exhibits a distinct hydrophobic bond between the two end C-atoms. These atom-pair correlations are intrinsically measurable by X-ray and neutron diffraction.<sup>53</sup> A natural view of the present case is that the chain molecule carries this hydrophobic pair into solution and permits them to find one another with reasonable frequency. The underlying assumption is that the hydrophobic interaction is sufficiently local that the effects of the supporting chain molecule are secondary. This is a reasonable assumption that can be experimentally tested by variation of the chain length and capping groups. Nonetheless, the present realization is consistent with the view that hydrophobic interactions are typically expressed in the context of other effects in micelles, membranes, and the structure of soluble proteins.

Two distinct sets of calculations were combined to obtain the present results: firstly, parallel tempering<sup>54</sup> to establish the overall structure of  $P(r)$  and, secondly, windowing<sup>60</sup> to achieve satisfactory spatial resolution in the interesting loop-closure regime,  $r < 0.7$  nm. Direct observation of the contact feature would rest on a few percent of the parallel-tempering data set, *i.e.*, about 2% of the molecules are in loop-closure configurations. The overall parallel-tempering results permit evaluation of the undetermined scale factor (zero of the potential of the average forces) for the windowing results.

The shape of  $P(r)$  in the loop-closure region is strikingly similar to predicted pair distributions for model inert gases in water.<sup>48,61,62</sup> On the basis of the stratified evaluation of  $P(r)$ , the most probable  $-\text{CH}_3 \cdots \text{CH}_3-$  hydrophobic bond length is about 0.4 nm. The slight shoulder on the large- $r$  side of the principal maximum of  $P(r)$  is a remnant of a RISM cusp<sup>63</sup> and reflects the

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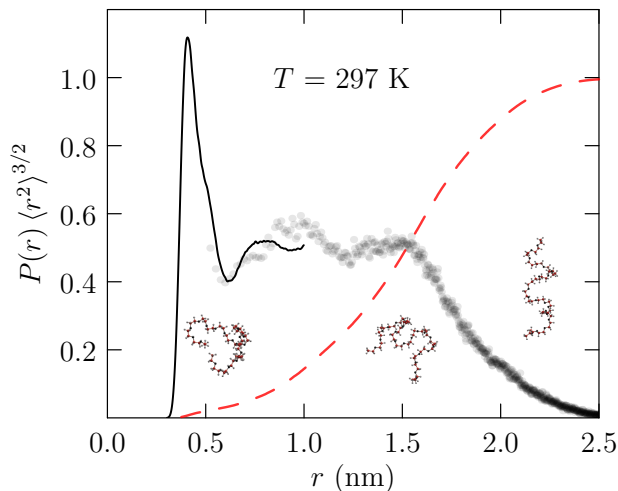


FIG. 1. Probability density  $P(r)$  for radial displacement of end-C atoms of  $\text{CH}_3\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{20}\text{CH}_2\text{CH}_3$  in aqueous solution, with the cumulative distribution  $\int_0^r P(x)4\pi x^2 dx$  (dashed line). The dots were obtained by parallel tempering<sup>54</sup> over times of 20 ns/replica with 32 temperatures spanning 256–550 K, using AMBER 10<sup>55</sup> with the same molecule numbers and cubical volume  $V = 32.254 \text{ nm}^3$  for each replica. Monte Carlo swaps of configurations had an overall success rate of 23%. The SPC/E model treated the 1000 water molecules<sup>56</sup>, and an extended atom model with the Generalized Amber Force Field (GAFF) was used for the single chain molecule.<sup>57</sup> The solid line is a stratified recalculation of the loop-closure feature, adopting a harmonic potential for the radial displacement coordinate covering the range of 0.3–1.0 nm uniformly with 15 windows, then reconstituting  $P(r)$  on that range with a weighted histogram method.<sup>58–60</sup> This second set of calculations lasted 20 ns/window. The undetermined multiplicative constant in the stratified calculation of  $P(r)$  is adjusted to match the direct observation from the parallel tempering in the region of the first minimum. The left vertical axis is non-dimensionalized with the observed  $\langle r^2 \rangle^{1/2} = 1.56 \text{ nm}$ .

ethyl end-capping.

A direct comparison with results for  $\text{CH}_4 \cdots \text{CH}_4$  obtained with the same models and stratification methods (FIG. 2) shows good agreement in the placement of the principal peak, and the large- $r$  shoulder of the principal peak is more obvious. Contact pairing configurations are more prominent than solvent-separated ones in both of these cases, supporting a standard view of this hydrophobic interaction. A separate evaluation (not shown) of the solubility of  $\text{CH}_4(\text{aq})$  with these models compares well with experiment. This substantiates the view that this model solute naturally represents hydrophobic methane. These probability densities differ in outer shells due to detailed differences between the solvent and the chain molecule medium.

It is natural also to compare these pdfs with what would be obtained if the solvent were absent (FIG. 3). The comparison shows that water swells this soluble chain molecule (FIG. 1), as is the case also with aqueous

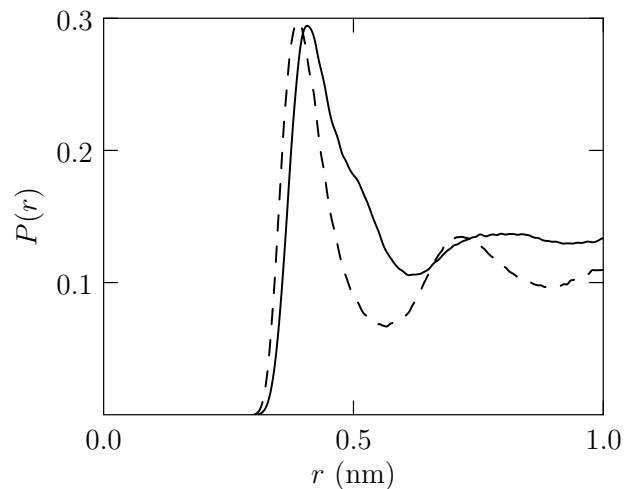


FIG. 2. Comparison of the probability density of FIG. 1 (solid curve) with results from the  $\text{CH}_4 \cdots \text{CH}_4$  potential of mean force obtained with the same models and stratification methods (dashed curve) as FIG. 1. The latter result is in good agreement with a previous simulation evaluation for a similar model.<sup>50</sup> The multiplicative constant required for this comparison was adjusted informally for agreement in the region of the maxima of these two results.

dilution of a PEO melt.<sup>64</sup> This supports the view that the chain molecule globule might have a secondary effect on the hydrophobic interaction which is of first interest here.

Note that the boundary between the low-extension  $r < \langle r^2 \rangle^{1/2}$  region and a high-extension region is definite (FIG. 1). On the basis of the similarity with the results for FIG. 2, the end-cap pair appears to be shielded within a uniform fluid environment in the low-extension region. Furthermore, end-caps are more concentrated in the smaller-volume polymer globule of FIG. 3 than in the larger-volume polymer globule of FIG. 1. The non-dimensionalized probability densities incorporate that distinction without which the maximum probabilities would differ by nearly a factor of three. This point is consistent with the observation from FIG. 2 that if the probability densities there match roughly at large- $r$ , then the maximum values also approximately match.

The high-extension  $r > \langle r^2 \rangle^{1/2}$  tail of the probability density (FIG. 1) is reasonably described by a linear-response behavior

$$-\frac{d}{dr} \ln P(r) \approx a + br. \quad (1)$$

A Gaussian model for  $P(r)$  at high-extension would not be centered on the origin, however, and in that sense a traditional Gaussian model would be unsatisfactory in the high-extension region.

Simple characterizations of hydrophobic effects, *i.e.* whether they are attractive or repulsive, depend on the specific properties examined and the comparisons made. For example, well-developed theoretical analyses<sup>49</sup> show

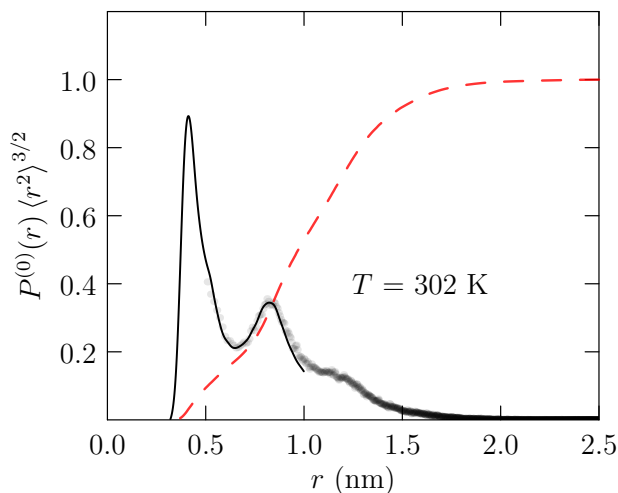


FIG. 3. As in FIG. 1 but without water present. The left vertical axis is non-dimensionalized with the observed  $\langle r^2 \rangle^{1/2} = 1.06$  nm.

that hydrophobic interactions can be repulsive for the osmotic second virial coefficient. Other comparisons illuminate different aspects of hydrophobic interactions. Sometimes hydrophobic interactions are judged by adopting another solvent that provides a natural comparison to the case of the water medium.<sup>65</sup> Sometimes it is most direct to judge hydrophobic interactions by comparison with standardly hydrophilic solutes, polar, H-bonding, or ionic species in water. We propose that identification of an experimentally accessible case that also permits detailed molecular theory and computation should assist in resolving such alternatives.

Though this hydrophobic interaction is intrinsically measurable, analysis of X-ray and neutron diffraction experiments on such systems will require specific and thorough support from molecular simulations, as is the current practice.<sup>53,66</sup> Isotopic substitution and labeling will be essential. Consideration of fluorinated caps such as  $-\text{CF}_3$ <sup>67,68</sup> should make this hydrophobic interaction more prominent yet.<sup>47</sup>

The chain molecules considered here have broad technological interest because of their biocompatibilities.<sup>69</sup> They are also intrinsic to the dispersant materials used in response to oil spills.<sup>70</sup> Small angle neutron scattering<sup>71</sup> and fluorescence and light-scattering studies<sup>72</sup> have shown that the aqueous solution interactions of  $(-\text{OCH}_2\text{CH}_2-)_n$  polymers are sensitive to the end-capping of the chains. The importance of end-effects is also supported by the sensitivity of solution phase diagrams to the  $(-\text{OCH}_2\text{CH}_2-)_n$  lengths.<sup>73,74</sup> These observations suggest hydrophobic bonding of the  $-\text{CH}_3$  caps, and that manipulation of capping groups might help in understanding hydrophobic interactions on a molecular scale<sup>45</sup> by exhibiting a localized hydrophobic bond.

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